CHAPTER 7

SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

Chronic kidney disease (CKD) is relatively common, affecting 10-15% of the global population, and devours a considerable proportion of health care budgets worldwide. Although the development of pharmacological agents targeting the renin-angiotensin-aldosterone system in the 80s en 90s of the previous century have resulted in the possibility to delay CKD progression, this delay comes down to just a modest postponement of end-stage renal disease and its cardiovascular complications, i.e a postponement of approximately 4 months (Figure 1) as compared to regular antihypertensive treatment. This applies to ACE inhibitor and ARB alike.

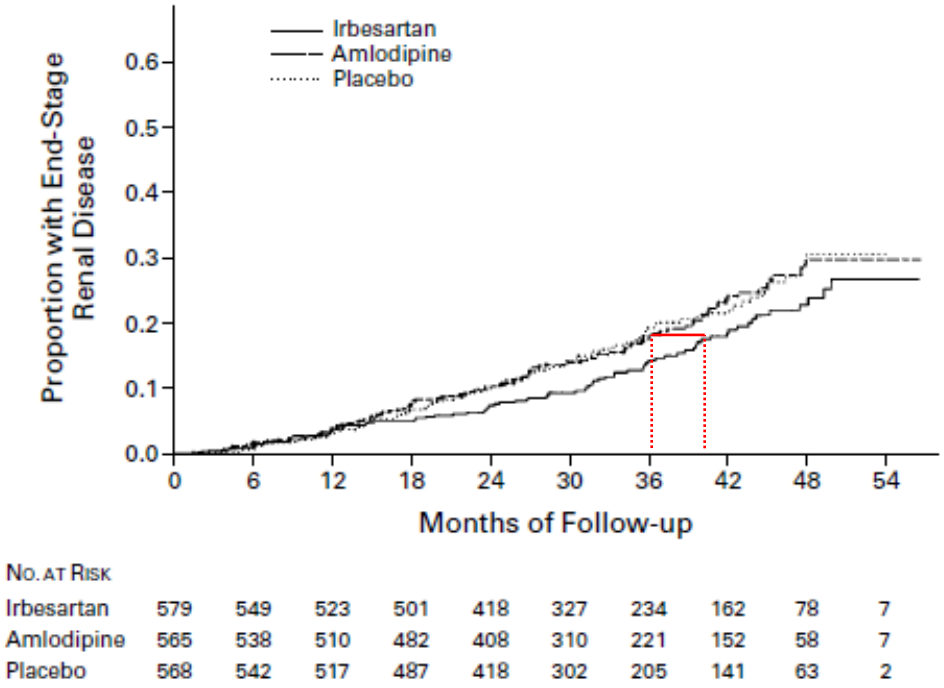

Figure 1. Cumulative proportion of patients reaching end-stage renal disease in the IDNT trial. Treatment with irbesartan delayed the progression towards end-stage renal disease by approximately 4 months compared with the placebo or amlodipine groups, for example from 36 to 40 months of follow-up, as indicated by the dashed lines. Reproduced from Lewis EJ et al.[1]

Disappointingly, since the introduction of ACE inhibitors and ARBs for renoprotection in the 1990's, no other therapy has convincingly been able to further retard progression of CKD. Similarly, no effective specific therapies to prevent the excess of cardiovascular disease have been identified in CKD patients. Considering the excessively high cardiovascular risk in renal patients, in particular in patients end stage renal failure (Figure 2) this remains a major challenge.

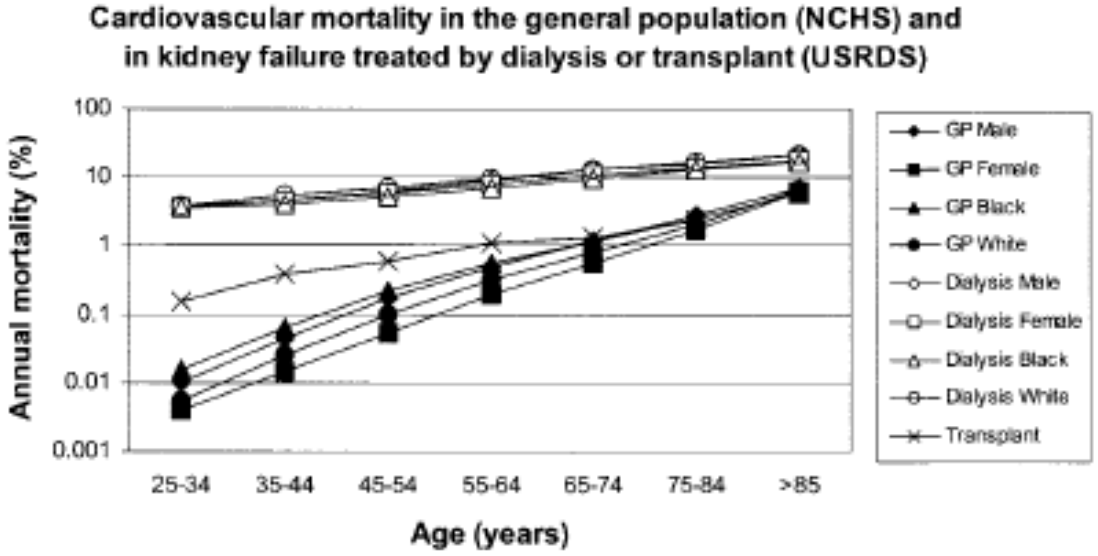

Figure 2. Cardiovascular mortality defined by death due to arrhythmias, cardiomyopathy, cardiac arrest, myocardial infarction, atherosclerotic heart disease, and pulmonary edema in the general population compared with dialysis patients and renal transplant recipients (USRDS data). Data are stratified by age, race, and sex. Reproduced from RN Foley et al[2].

Increasing evidence indicates that not only conventional cardiovascular risk factors, like hypertension, smoking and high cholesterol are involved in the excess cardiovascular risk in renal patients, but that risk factors specific to renal disease are important as well. Among these risk factors, deregulations of phosphate metabolism stand out as important candidate-pathways for the increased cardiovascular risk in

CKD. Specifically, deregulations in serum phosphate and the phosphaturic hormone fibroblast growth factor 23 (FGF23) have strongly been linked with a higher cardiovascular risk, and may be specific targets for intervention in CKD and after kidney transplantation. An important question is whether (partial) improvement of phosphate metabolism after kidney transplantation is responsible for the reduced cardiovascular mortality risk compared with the dialysis population and whether a further improvement in phosphate metabolism may increase cardiovascular prognosis. Although drugs and dietary strategies to target hyperphosphatemia are available, it is still unknown how to specifically target FGF23, and whether these strategies may actually improve patient morbidity and mortality.

In chapter 2, we reviewed the literature on phosphate homeostasis, focusing on the clinical implications of its deregulation after renal transplantation. Early after transplantation, serum phosphate levels decline rapidly in response to restored renal function in the context of residually high FGF23 and PTH levels. This may result in deep hypophosphatemia requiring phosphate supplementation. In contrast, later after transplantation, when renal function has declined, patients may require phosphate-lowering therapy to normalize serum phosphate, and possible also FGF23 levels. Pharmacological and dietary therapies aiming to improve phosphate metabolism are available to renal transplant recipients, yet levels of phosphate and, mostly, FGF23 may still increase strongly with declining graft function as in CKD. Analogous to the pre-transplant CKD setting, it seems prudent to aim for normalization of serum phosphate and FGF23 levels in renal transplant recipients, although hard endpoint data confirming the clinical relevance of reaching these targets in the transplant population are lacking.

In chapter 3, we investigated fibroblast growth factor 23 (FGF23) as a potential predictor of cardiovascular mortality in a large cohort of kidney transplant recipients. Several studies had previously linked a high FGF23 level with adverse cardiovascular outcomes in pre-transplant CKD patients. In this study we found strong associations between FGF23 and cardiovascular markers, such as NT-proBNP, MR-proANP and copeptin. We evaluated the association of FGF23 levels with outcomes in Cox regression models, adjusted for several potential confounders, and found that FGF23 levels were strongly and consistently associated with a higher risk of both cardiovascular and all-cause mortality, both independent of renal function. The association of FGF23 with graft failure was lost after adjustment for renal function.

The potential role for a high FGF23 level as an independent predictor of cardiovascular outcomes observed in chapter 3 underlines the need for novel interventions lowering FGF23 in renal transplant recipients. Although classical strategies including a phosphate-restricted diet and phosphate binders are at least partly successful to lower phosphate and FGF23 levels, as reviewed in chapter 2, additional strategies are required. To design and prioritize anti-FGF23 strategies, it would be useful, therefore, to first have an overview of the determinants of FGF23 in the clinical setting, including clinical characteristics as well as dietary factors. Moreover, given the strong associations observed between parameters of inflammation and deregulations in FGF23, it would be interesting to include in such an analysis nutrients that, by anti-inflammatory effects have been implicated to have cardioprotective properties, such as N-3 polyunsaturated fatty acids (N-3 PUFAs) [3-4].

In chapter 4, we analysed the clinical and dietary determinants of FGF23 in a cross-sectional study in stable kidney transplants, including assessment of the possible association of intake of fish (as a dietary source of PUFAs) and the N-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with serum FGF23 levels. This cross-sectional analysis revealed that, indeed, higher intake of fish and dietary N-3 PUFAs (EPA-DHA) were associated with lower levels of circulating FGF23, independent of potential confounders. Interestingly, in patients with a reduced graft function (eGFR the renal function <60 mL/min/1.73 m^2) the association between FGF23 levels and N-3 PUFAs was most pronounced. Based on the findings from this observational study, we subsequently performed a prospective study in an animal model of CKD.

In chapter 5, we investigated the effect of N-3 PUFAs supplementation in a mouse model of renal inflammation. After inducing inflammation by adenine-enriched chow administration, we observed the development of intra-renal inflammation (higher levels of renal HMOX, IL-6 and TGF-β expression), accompanied by reduced renal expression of klotho (an essential co-factor for the specific receptor for FGF23) and renal fibrosis. Conversely, when animals were treated with fish oil-enriched chow (rich in N-3 PUFAs), the intra-renal inflammation as well as renal klotho expression and collagen deposition were restored to control levels, suggesting that the N-3 PUFAs supplementation had strong beneficial effects on renal klotho expression, and prevented renal inflammation and fibrosis. These positive and exciting results, and the known inverse relationship between renal klotho and FGF23 levels suggest that by restoring renal klotho expression, PUFAs may also beneficially impact on circulating FGF23

levels. To address this in patients, we measured FGF23 levels in a posthoc analysis of the Alpha Omega trial participants with impaired renal function (eGFR <60 ml/min/1.73 m2). As reported in **chapter 6**, we found no effects of PUFA supplementation on FGF23 in this population. Although PUFA supplementation provided a small protective effect on renal function in a previous study, this did not translate into a lower FGF23 level in the current sub-cohort of the Alpha Omega trial[5]. However, the PUFA dose was relatively low, and due to the post-hoc design, investigation of the effect of higher doses was not possible.

Future directions

Given the strong and consistent association of high plasma FGF23 level with an increased risk of cardiovascular mortality, we can conclude that FGF23 should be considered a promising candidate-target to improve cardiovascular outcome in renal transplant recipients and the CKD population. The observation that N-3 PUFA and fish intake was inversely associated with FGF23 levels in renal transplant recipients (chapter 4), and the capacity of fish oil supplementation to reduce renal damage in our animal study (chapter 5) call for further clinical studies addressing the potential cardiorenal protective effects of PUFA-based dietary interventions in CKD and transplant patients. The transplant population may be of particular interest, given the protective effect of fish-oil aginst rejection, as reported in the 1990's.

Although we could not demonstrate an effect of PUFA supplementation on FGF23 levels in post-myocardial infarction CKD patients in the Alpha Omega Trial, it is

important to note that the supplementation doses of EPA-DHA in this study were relatively low, and may therefore have not been sufficient to result in detectable reduction of FGF23 levels. Therefore, further intervention studies using higher doses of EPA-DHA are needed to confirm the present data.

Management of phosphate levels has a long tradition in nephrology, and involves both dietary phosphate restriction and intestinal phosphate binders[6]. Yet, few data are available on the effect of phosphate correction on FGF23 levels, in CKD patients and kidney transplant recipients. However, as patients with hyperphosphatemia have higher phosphate intake than patients with hypophosphatemia, so dietary phosphate restriction could be useful to avoid or correct high phosphate levels and hence prevent increases in FGF23 levels[7]. To this purpose, special attention on inorganic phosphate intake, commonly added as preservative in processed foods, should be paid since its intestinal absorption is much higher than organic phosphate and could exert a bigger impact on phosphate and FGF23 levels[8]. The conventional phosphate binders includes, mainly, sevelamer and calcium salts[6]. Some pharmacological novelties have been studied and are still under (pre-) clinical investigation. Nicotinamide, an inhibitor of intestinal NaPi-2b transporters has presented positive results on decreasing phosphate levels in CKD patients[9-10]. In line, tenapanor, an inhibitor of the intestinal sodium-hydrogen exchanger 3, has also shown to decrease phosphate uptake as well as ectopic calcification in an experimental model of CKD[11]. Although such drugs are still not established therapies to decrease phosphate levels, in the future, nicotinamide and tenapanor may contribute to the management of deregulated phosphate homeostasis in both CKD and transplant patients. Recently, an experimental study used a monoclonal FGF23 antibody to

evaluate the impact of chronic FGF23 neutralization on CKD-MBD. However, the neutralization of FGF23 increased serum phosphate levels and contributed to increase mortality in that CKD-MBD rat model[12].

Although much more is known about phosphate/FGF23 metabolism, there are two important questions to be answered: Firstly, what is the best approach to improve phosphate metabolism in both CKD and renal transplantation? Secondly, would normalization of phosphate metabolism reduce cardiovascular morbidity and mortality, and contribute to long-term preservation of renal function, in CKD and after kidney transplantation? The answers to these questions will provide a basis for further improvement of cardiorenal prognosis in CKD and transplanted patients.

References

1. Lewis E.J. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med*. 345:851-860 (2001).

2. Foley R.N., Parfrey P.S., Sarnak M.J. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis*. 32 112–119 (1998).

3. Moreno, J.A. *et al*. The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. *J Am Soc Nephrol*. 22,1315-1325 (2011).

4. Mendoza J. e*t al*. Fibroblast growth factor 23 and Inflammation in CKD. *Clin J Am Soc Nephrol.* 7, 1155-1162 (2012).

5. Hoogeveen E.K. *et al.* Effect of omega-3 fatty acids on kidney function after myocardial infarction: the Alpha Omega Trial. *Clin J Am Soc Nephrol*. 9, 1676-1683 (2014).

6. Navaneethan SD, Palmer SC, Craig JC, Elder GJ, Strippoli GF. Benefits and harms of phosphate binders in CKD: a systematic review of randomized controlled trials. *Am J Kidney Dis*. 54, 619-637 (2009).

7. Rho, M. R. *et al*. Evaluation of nutrient intake in early post kidney transplant recipients. *Clin. Nutr. Res.* 2, 1-11 (2013).

8. Kalantar-Zadeh, K. *et al*. Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* 5, 519-530 (2010).

9. Maccubbin, D., Tipping, D., Kuznetsova, O., Hanlon, W. A. & Bostom, A. G. Hypophosphatemic effect of niacin in patients without renal failure: a randomized trial. *Clin. J. Am. Soc. Nephrol.* 5, 582-589 (2010).

10. Ix, J. H., Ganjoo, P., Tipping, D., Tershakovec, A. M. & Bostom, A. G. Sustained hypophosphatemic effect of once-daily niacin/laropiprant in dyslipidemic CKD stage 3 patients. *Am. J. Kidney Dis.* 57, 963-965 (2011).

11. Labonte, E. D. *et al*. Gastrointestinal Inhibition of Sodium-Hydrogen Exchanger 3 Reduces Phosphorus Absorption and Protects against Vascular Calcification in CKD. *J. Am. Soc. Nephrol.* 17 *Epub ahead of print* (2014).

12. Shalhoub, V. *et al*. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. *J. Clin. Invest*. 122, 2543–2553 (2012).